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13. ABSTRACT *(Maximum 200 words)*

During hibernation, mammals rely heavily on lipid stores to provide the fuel necessary to survive the winter. Pyruvate dehydrogenase kinase isozyme 4 (PDK-4) plays a key role in gating carbohydrate catabolism and allowing the switch to lipid metabolism. Earlier we reported that PDK-4 was up-regulated in the heart of the thirteen-lined ground squirrel during hibernation. PDK-4 is encoded by the nuclear genome and acts by phosphorylating pyruvate dehydrogenase (PDH) thus attenuating its activity. PDH catalyzes the oxidation of pyruvate to acetyl CoA, the first irreversible step in glycolysis. Northern blot analysis shows that PDK-4 message is upregulated 20-fold in heart and 5-fold in skeletal muscle during hibernation. Unlike PDK-4, two other differentially expressed genes located in the mitochondrial genome are down-regulated during hibernation in heart and one is down-regulated in skeletal muscle. These genes encode NADH dehydrogenase 5 (NDH5) and NADH dehydrogenase 6 (NDH6), two subunits of NADH ubiquinone-oxidoreductase (complex I).

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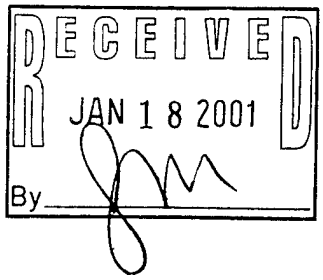

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Enclosure 3

b. FINAL PROGRESS REPORT

Regulation of Genes Controlling Carbohydrate Metabolism in the Heart of a Hibernating Mammal

Matthew T. Andrews, P. I.

Statement of the problem studied

Mammals in deep hibernation have an observed respiratory quotient (RQ) of 0.7 (Lyman, 1982) RQ is a unit-less value representing the moles of CO_2 respired per moles of O_2 consumed. A value of 1.0 indicates combustion of carbohydrate; however, an RQ of 0.7 indicates that fat is the major substrate for energy production. This fuel selection of fat over carbohydrate seen during hibernation also occurs in starvation, when conservation of glucose for utilization by the brain is essential for survival and is controlled by the phosphorylation of pyruvate dehydrogenase (Randle, 1986) Since hibernating ground squirrels can survive 5-6 months without feeding, mechanistic insight into the activation of genes such as PDK-4 could provide a better understanding of how nutrient-gene interactions influence an animal's capacity to cope with the effects of starvation. Recent experiments with rats have shown that starvation for a period of 12 to 24 hours results in a sharp increase in heart PDK-4 mRNA (Wu, 1998) As seen with ground squirrel hearts at spring arousal, this up-regulation of PDK-4 in rats is reversible as heart PDK-4 mRNA returns to control levels upon re-feeding.

It has been known for over 30 years that diabetes, like starvation, causes an overall reduction in cardiac glucose oxidation (Bleehen, 1954; Morgan, 1961; Morgan, 1961) An examination of working hearts in diabetic pigs has shown that the major defect in carbohydrate catabolism was not in glycolysis, but in the oxidation of pyruvate by pyruvate dehydrogenase (Hall, 1996). Experiments with rat hearts (Wu, 1998) have shown that high levels of PDK-4 expression were induced in hearts of animals rendered diabetic by treatment with streptozotocin - an antibiotic that destroys the insulin producing capacity of the pancreas. As with starvation and spring arousal, this induction is reversible as high levels of both PDK-4 protein and mRNA in the hearts of diabetic animals are reduced to control levels upon treatment with insulin (Table 1). As a positive regulator of glucose uptake and utilization, it has been a long-standing observation that insulin inhibits heart PDK activity (Hutson, 1978) Only now is it evident that this effect is mediated by regulating the expression of the gene encoding PDK-4.

Table 1. Conditions affecting PDK-4 expression in the heart

PDK- 4 ↑ - stable increase in PDK-4 mRNA levels

1. Hibernation (ground squirrel; Andrews *et al.*, 1998)
2. Starvation (rat; Wu *et al.*, 1998)
3. Diabetes (rat; Wu *et al.*, 1998)

PDK- 4 ↓ - reversal of PDK-4 mRNA to control levels (refs. for each numbered item same as above)

1. Spring arousal
2. Starvation + re-feeding
3. Diabetes + insulin

Summary of the most important results

The thirteen-lined ground squirrel, a characteristic deep hibernator, slows its metabolism and drops its body temperature to survive the harsh winter months when food is scarce. To survive without eating, meticulous regulation of energy resources is essential during hibernation. In the heart of the thirteen-lined ground squirrel, this regulation is accomplished by changes in gene expression that result in a switch from carbohydrate catabolism to fatty acid oxidation. Using a PCR-based subtractive hybridization scheme, three genes encoding mitochondrial proteins were found to be differentially expressed in the hearts of hibernating ground squirrels. One gene, pyruvate dehydrogenase kinase isozyme 4 (PDK-4), is nuclear encoded and is upregulated during hiberation. PDK-4 acts by phosphorylating pyruvate dehydrogenase (PDH) and attenuating its activity. PDH catalyzes the oxidation of pyruvate to acetyl CoA, the first irreversible step in glycolysis. Northern blot analysis shows that PDK4 message is upregulated 20-fold in heart and 5-fold in skeletal muscle during hibernation.

Unlike PDK-4, the two remaining differentially expressed genes are located in the mitochondrial genome and are down regulated during hibernation in heart and ndh6 is down in skeletal muscle. These genes encode NADH dehydrogenase 5 (NDH5) and NADH dehydrogenase 6 (NDH6), two subunits of NADH ubiquinone-oxidoreductase (complex I). Complex I is a large multi-protein component of the mitochondrial electron transport chain containing more than 30 subunits and is the first carrier of electrons in the system. NDH5 is transcribed from the mitochondrial

H-strand along with 11 other protein coding genes. Although NDH5 is down regulated, genes flanking either side of it on the H-strand are not. This difference in expression suggests a specific mechanism for reduction of NDH5 message. NDH6, on the other hand, is the only L-strand encoded protein. Its open reading frame partially overlaps with NDH5. Down-regulation of NDH5 and NDH6 in combination with the upregulation of PDK-4 is concordant with the observed reduction in mitochondrial state 3 respiration during hibernation in mammals.

In the mitochondrial genome, the bi-directional promoter region regulates transcriptional control for all genes in the mitochondria. Because ndh6 is the only protein encoding gene on the L strand, a reduction in L strand initiation would curtail ndh6 mRNA. Transcriptional initiation, however, cannot explain the reduction in ndh5 mRNA. Ndh5 is down-regulated, but flanking genes are not. The mechanism for the down-regulation of a single gene on the H strand is still unknown.

Differences in tissue-specific expression patterns for ndh5 and ndh6 imply distinct mechanisms for control. Decreased expression of ndh6 is not muscle-type specific. In an individual animal, ndh6 is down-regulated in both heart and skeletal muscle suggesting an organismal signal or factor causing the abated ndh6 expression during hibernation. Ndh5, on the other hand, is down-regulated in heart but not skeletal muscle. This divergence implies a tissue-specific signal or factor controlling its expression.

List of all publications and technical reports

Published articles:

Squire, T.L. and Andrews, M.T. (2000) Genetic control of carbon utilization during hibernation: mechanistic considerations, in *"Life in the Cold"* (G. Heldmaier, S. Klaus, M. Klingenspor, Eds.) pp. 325-337, Springer-Verlag, Berlin.

Abstracts:

Buck, M.J. and Andrews, M.T. (2000) Coordinated regulation of PDK-4 in cardiac, skeletal muscle, and adipose tissue during hibernation in the thirteen-lined ground squirrel. Eleventh International Hibernation Symposium, Jungholz, Austria, August 13-18, 2000.

Squire, T.L., Bauer, V.W., Lowe, M.E., and Andrews, M.T. (2000) Genetic regulation of lipolysis during hibernation. Eleventh International Hibernation Symposium, Jungholz, Austria, August 13-18, 2000.

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UNDERGRADUATE RESEARCH PROJECTS DIRECTED

Luis Miguel Gonzalez, 1999-2000

Report of inventions

None

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